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Plasmid profile, serogrouping, and auxotyping of Neisseria gonorrhoeae isolates from Africa

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SUMMARY The plasmid patterns of 90 isolates of *Neisseria gonorrhoeae* (including 39 penicillinase-producing strains) originating from various countries in Africa were determined. Serogrouping by coagglutination and auxotyping were used to characterise the isolates. The 4·4-megadalton plasmid was present in seven isolates out of 39 penicillinase-producing strains, two of which occurred with a conjugative 24·5-megadalton plasmid. The African strains were predominantly serogroup WI and wild type. Arginine-dependent isolates were as common as proline-dependent types.

Introduction

New plasmid patterns of *Neisseria gonorrhoeae* are occurring, and the geography of previously reported plasmid patterns is changing.[1-4] Jephcott *et al*[1] found an endemic focus of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) with 4·4-megadalton (mdal) plasmid in Britain. The combination of 3·2 mdal plasmid and a 24·5 mdal conjugative plasmid has recently been detected in PPNG isolates from Canada,[2] the Netherlands,[3] and Britain.[4] The recent report[5] of the detection of 4·4 mdal plasmid in PPNG isolates from Nigeria raises a fundamental question of whether such isolates with 4·4 mdal plasmid were imported into West Africa or were in fact indigenous strains which were unidentified in the other previous studies,[6 7] probably because of the limited number of PPNG isolates from Africa. Auxotyping technique[8 9] and the new serogrouping method[10-13] based on antigens designated "W" offer potentially valuable tools for further evaluation of the epidemiology of gonococcal infections from various geographical areas.

In the present study we examined the plasmid content of *N gonorrhoeae* isolates sent to our laboratory from various countries in Africa. In addition, the serogroup and auxotype of each of the gonococcal isolates were determined.

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Material and methods

Ninety isolates of *N gonorrhoeae,* including 39 strains of PPNG isolated from patients in various African countries, were studied. The identity of all the gonococcal isolates was reconfirmed in our laboratory at the Center for Disease Control. The β-lactamase production of each isolate was retested by the chromogenic cephalosporin[14] and starch paper technique.[15]

Plasmid deoxyribonucleic acid from the cleared lysate of each gonococcal isolate was precipitated with ethanol and subjected to agarose gel electrophoresis.[16] Plasmids of known molecular weight were included as standards. Nutritional growth requirements of each gonococcal isolate were determined by auxotyping techniques.[8 9]

Each gonococcal isolate was serogrouped using the coagglutination methods.[11-13] Reagents were kindly provided by Dr Joan S Knapp (University of Washington, Seattle, USA). The reagent consisted of staphylococci sensitised with hyperimmune rabbit antisera to *N gonorrhoeae.* A drop of the boiled suspension of the gonococcal cells was mixed with a drop of the reagent. A positive coagglutination reaction was evident after the mixture had been rotated for two minutes. The association of *N gonorrhoeae* isolates with serogroup and geographical source was assessed by the Loglinear model, χ^2 analysis.

Results

The 90 isolates tested included all the 39 African PPNG strains available in our laboratory from 1977 to

April 1982. These included isolates from the Central African Republic (6 PPNG/16 non-PPNG), Cameroon (0/3), Ethiopia (0/6), Ghana (7/7), Ivory Coast (1/0), Kenya (5/11), Nigeria (16/6), South Africa (0/2), Zambia (2/0), and Zaire (2/0).

The 4·4 mdal plasmid was found in seven of 39 PPNG isolates examined; two of these also carried a conjugative 24·5 mdal plasmid (table I). The remaining 32 PPNG strains contained the 3·2 mdal plasmid, but none carried the 24·5 mdal plasmid. The cryptic plasmid (2·6 mdal) was present in all PPNG isolates and in 49 of the 51 non-PPNG isolates. Two of the non-PPNG isolates contained the conjugative plasmid. The wild type was predominant in both PPNG and non-PPNG isolates (table II). Only 10 of the 90 strains tested were arginine dependent.

Thirty-four of 39 PPNG isolates were serogroup WI. The African isolates of PPNG were significantly associated with serogroup WI ($\chi^2 = 19·23$, $p = 0·0001$) (table III). Only 20 of the 51 non-PPNG strains were serogroup WII. All the four PPNG strains from West Africa harbouring the 4·4 mdal plasmid belonged to serogroup WI, while all the three strains from other African countries harbouring 4·4 mdal plasmid belonged to serogroup WII. Only two strains out of 32 PPNG isolates having 3·2 mdal plasmid belonged to serogroup WII (table IV).

TABLE I *Distribution of plasmids in PPNG from African sources*

Geographical source	No of strains tested	No of isolates having plasmids of molecular weight (megadaltons)*: 3·2	4·4
Central African Republic	6	6	—
Ghana	7	5	2
Ivory Coast	1	1	—
Kenya	5	2	3†
Nigeria	16	14	2
Zambia	2	2	—
Zaire	2	2	—
Total	39	32	7

*All isolates contained a 2·6 mdal plasmid.
†Two isolates also contained a 24·5 mdal plasmid.
PPNG = penicillinase-producing *N gonorrhoeae*

TABLE III *Serogrouping of African isolates of N gonorrhoeae by source of recovery and presence or absence of β-lactamase production*

	No (%) of isolates: Serogroup WI	Serogroup WII	Probability
Source of recovery:			
West Africa	30(77)	7(23)	0·035
Other African countries	24(45)	29(55)	
Strain type:			
PPNG	34(87)	5(13)	<0·0001
Non-PPNG	20(39)	31(61)	

PPNG = penicillinase-producing *N gonorrhoeae*

TABLE IV *Relationship between auxotype and serogroup of PPNG by plasmid type from African sources*

Auxotype	No of isolates with 3·2 mdal plasmid (No with 4·4 mdal plasmid) Serogroup WI	Serogroup WII	Total
Wild type	20	0(1)	20(1)
Pro⁻	4*(4)	1(2)	5(6)
Arg⁻	6	1	7
Total	30(4)	2(3)	

*One isolate required both proline and arginine
PPNG = penicillinase-producing *N gonorrhoeae*

Discussion

It is noteworthy that a conjugative 24·5 mdal plasmid was found in both PPNG and non-PPNG isolates from African sources in this study. The only two PPNG strains harbouring the conjugative plasmid carried the 4·4 mdal penicillinase plasmid; one of the strains was proline requiring while the other was a wild type, but both belonged to serogroup WII. Bygdeman *et al*[10] reported that all the six PPNG strains from African sources belonged to serogroup WI, even though two of the six strains were arginine requiring; three were wild type and one was proline requiring. Although only six African PPNG strains were reported on by Bygdeman *et al*,[10] the results accord

TABLE II *Auxotypes of PPNG and non-PPNG from African sources*

Source	Strain	No of isolates tested	No of isolates with requirements for: Wild-type (non-requiring)	Pro	Arg	Pro Arg	Isoleucine
West Africa*	PPNG	24	11	6	6	1	0
	Non-PPNG	13	4	7	2	0	0
Other African countries†	PPNG	15	10	4	1	0	0
	Non-PPNG	38	25	10	1	1	1

Pro = proline; Arg = arginine; PPNG = penicillinase-producing *N gonorrhoeae*
*West African countries include Ghana, Ivory Coast, and Nigeria
†Cameroon, Central African Republic, Ethiopia, Kenya, South Africa, Zambia, and Zaire

with our own findings that PPNG from African sources belong to serogroup WI while the auxotype could vary between the wild type, proline-requiring, and arginine-requiring types. Since the four PPNG strains harbouring 4·4 mdal plasmid from West Africa all belonged to serogroup WI and shared the same antigenic specificities within the serogroup, we conclude that these strains are indigenous to West Africa, while the three PPNG strains with 4·4 mdal plasmid from other African countries in serogroup WII were imported into those countries.

It is interesting to note that six out of seven PPNG strains carrying 4·4 mdal plasmid were proline requiring and the one remaining strain was wild type. This finding is in accord with previous reports[3 6] that 4·4 mdal plasmid is found predominantly in proline-requiring gonococcal isolates. The problem of stability of the 4·4 mdal plasmid in West African PPNG strains requiring proline and belonging to serogroup WI might be the factor determining the low prevalence of 4·4 mdal plasmid in PPNG isolates from West Africa.

We conclude that serogrouping and auxotyping of *N gonorrhoeae* are valuable adjuncts to the epidemiological studies of PPNG infections. Furthermore, our data show that all the different plasmid patterns except the combination of 24·5 mdal and 3·2 mdal plasmids reported from all other geographical areas are present in Africa.

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